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Review

$GABA_A$ receptor subtypes underlying general anesthesia

Robert P. Bonin [a], Beverley A. Orser [a,b,c,*]

[a] Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8
[b] Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada M5S 1A8
[c] Department of Anesthesia, Sunnybrook Health Science Center, Toronto, Ontario, Canada M4N 3M5

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Abstract

General anesthetics produce a constellation of behavioral responses and widespread neurodepression. Recent studies have implicated the γ-aminobutyric acid (GABA) subtype A receptor as a primary anesthetic target. During the past decade, considerable progress has been made in dissecting the behavioral effects of anesthetics according to the subunit composition of $GABA_A$ receptors. In this review, we describe how particular $GABA_A$ receptor subtypes expressed in different brain regions are critical for the expression of behavioral endpoints, such as amnesia, sedation, and hypnosis.

Keywords: Anesthesia; GABA; $GABA_A$ receptors; GABA modulators; THIP; Isoflurane; Etomidate

Contents

1. Overview

Anesthetics have been used to relieve human suffering since the beginning of recorded time. Dioscorides (40–90 AD) first coined the Greek term "anesthesia" to describe an unnatural sleep caused by wine produced from the mandrake plant. Since then, general anesthesia has evolved into a sophisticated therapeutic intervention supported by state-of-the-art technology. Whereas the clinical use of anesthetics has mushroomed, an understanding of the mechanisms of drug action has lagged behind. Recent studies have shown that general anesthesia is not a single phenomenon but rather a complex state comprising multiple components (sedation, amnesia, hypnosis, analgesia, and immobility). Moreover, the various components of the anesthetic state may be mediated by different receptor populations and neuronal pathways (Campagna et al., 2003). The current challenge is to identify which receptors mediate the desirable components of the anesthetic state.

A promising candidate receptor for many of the therapeutic actions of anesthetics is the γ-aminobutyric acid (GABA) subtype A receptor. Here, we summarize several recent discoveries related to the effects of anesthetics on $GABA_A$ receptors. These advances have included the identification of extrasynaptic $GABA_A$ receptors as sensitive targets for low concentrations of anesthetics. Other in vitro studies have shown that injectable anesthetics (e.g., etomidate) and volatile

* Corresponding author. Department of Physiology, Room 3318, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario, Canada M5S 1A8. Tel.: +1 416 978 0574; fax: +1 416 978 3687.
E-mail address: beverley.orser@utoronto.ca (B.A. Orser).

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anesthetics (e.g., isoflurane) interact with specific amino acid residues on $GABA_A$ receptor subtypes. The behavioral phenotype of genetically modified mice that express anesthetic-insensitive subunits supports the hypothesis that different $GABA_A$ receptors subtypes underlie different anesthetic effects.

This review focuses on $GABA_A$ receptors which are key targets that mediate most of the clinically important effects of intravenous anesthetics. The predominant role of $GABA_A$ receptors in inhaled anesthetic effects, particularly immobility, is less clear. The contribution of other receptor populations including NMDA receptors, potassium channels, HCN channels and voltage-gated sodium channels to the actions of inhaled anesthetics has been recently elsewhere (Hemmings et al., 2005; Sonner et al., 2003).

2. Anesthetics and $GABA_A$ receptor physiology

GABA is the major inhibitory neurotransmitter in the mammalian brain, and as many as one-third of all synapses are GABAergic (Bloom and Iversen, 1971). Most inhibition is mediated by $GABA_A$ receptors, which are chloride-permeable ligand-gated ion channels. Activation of $GABA_A$ receptors generally leads to an influx of chloride, hyperpolarization of the membrane, shunting of excitatory input, and reduced excitability of the neurons. The $GABA_A$ receptor itself is a heteropentameric complex composed of five different subunits. At least 19 mammalian genes encode for different $GABA_A$ receptor subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, φ, π and ρ_{1-3}), with additional diversity arising from alternative splicing (Macdonald and Olsen, 1994). The most common combination of subunits is α, β, and γ, in a ratio of 2:2:1 in most $GABA_A$ receptors. The γ subunit may be replaced by a δ or ε subunit, depending on the brain region. The subunit composition can dramatically alter the biophysical properties of the receptors and drug sensitivity (Barnard et al., 1998).

GABA mediates the majority of inhibition by generating fast, transient inhibitory postsynaptic currents (IPSCs; Fig. 1). $GABA_A$ receptors clustered at postsynaptic terminals are activated by a near-saturating concentration of GABA (Maconochie et al., 1994). This form of synaptic or "phasic" inhibition maintains high-fidelity neuronal communication and produces precise timing of action potentials and synchronization of neuronal populations (Cobb et al., 1995;Pouille and Scanziani, 2001). For many years, enhancement of fast synaptic inhibition was widely thought to be the primary mechanism underlying the actions of many GABAergic drugs.

Over the past decade, however, a persistent form of tonic inhibition has been identified in several brain regions. This tonic inhibitory conductance is generated by high-affinity, slowly desensitizing $GABA_A$ receptors that are activated by low ambient concentrations of GABA (Farrant and Nusser, 2005). Tonic inhibition is expressed in a manner specific to particular cell types and occurs throughout the mammalian brain (Kullmann et al., 2005). Tonic conductance has been identified in cerebellar granule cells (Brickley et al., 1996; Wall and Usowicz, 1997), the hippocampus (Bai et al., 2001;Nusser and Mody, 2002; Stell and Mody, 2002), thalamocortical relay

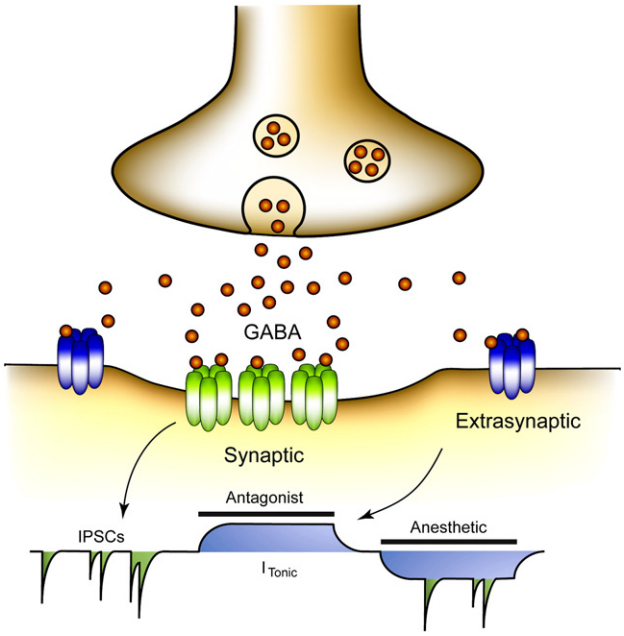

Fig. 1. Synaptic and extrasynaptic activation of γ-aminobutyric acid subtype A ($GABA_A$) receptors. Action-potential-dependent release of GABA into the synaptic cleft transiently activates $GABA_A$ receptors in the postsynaptic membrane. This generates inhibitory postsynaptic currents, which are typically of a brief duration due to GABA diffusion and uptake, and the desensitization of synaptic receptors. Extrasynaptic $GABA_A$ receptors are activated by low concentrations of GABA in the extracellular space arising from synaptic spillover or other non-synaptic release mechanisms. These receptors have low desensitization rates and can produce a continuous or "tonic" current. The tonic current is revealed by application of a $GABA_A$ antagonist, which inhibits the current. Many anesthetics potently enhance the tonic current at clinically-relevant concentrations.

neurons of the ventral basal complex (VB; (Belelli et al., 2005; Cope et al., 2005; Jia et al., 2005; Porcello et al., 2003), and the neocortex (Drasbek and Jensen, 2006; Salin and Prince, 1996). In the hippocampus, a tonic conductance is expressed by several cell types, including CA1 pyramidal neurons (Bai et al., 2001; Caraiscos et al., 2004a; Marchionni et al., 2007), granule cells in the dentate gyrus (Nusser and Mody, 2002), and hippocampal interneurons (Semyanov et al., 2003; Stell and Mody, 2002). This tonic conductance is activated by low ambient concentrations of GABA released via several mechanisms, including spillover from neighboring synapses and nonvesicular release mechanisms, such as reverse operation of GABA transporters (Cavelier et al., 2005). In the hippocampus, the tonic conductance is also activated by GABA released by action-potential-dependent vesicular mechanisms (Glykys and Mody, 2007). The tonic conductance has been shown to regulate neuronal excitability and information processing, both in vitro (Bonin et al., 2007; Brickley et al., 2001; Marchionni et al., 2007; Mitchell and Silver, 2003; Semyanov et al., 2003) and in vivo (Chadderton et al., 2004).

The importance of the tonic conductance to anesthetics actions was first demonstrated in the CA1 pyramidal neurons (Bai et al., 2001) and has since been shown for other cell types. For several reasons, extrasynaptic $GABA_A$ receptors that generate tonic conductance are predicted to be highly sensitive to anesthetics. First, these receptors are activated by low

ambient concentrations of GABA, and most anesthetics increase $GABA_A$ receptor affinity for agonist (Orser et al., 1998). Under conditions of low GABA occupancy, an increase in agonist affinity increases agonist binding and hence current amplitude. In contrast, postsynaptic $GABA_A$ receptors are activated by near-saturating concentrations of transmitter. This condition constrains a further increase in channel function through increased agonist binding. Also, extrasynaptic receptors desensitize to a lesser extent than their synaptic counterparts (Bai et al., 2001; Bianchi et al., 2002; Haas and Macdonald, 1999; Yeung et al., 2003). Anesthetic enhancement of GABAergic inhibition also appears to preferentially increase the tonic current over synaptic current in some brain regions. In direct comparisons of the effects of propofol, midazolam (Bai et al., 2001), etomidate (Cheng et al., 2006), and gaboxadol (Cope et al., 2005) on the tonic conductance and spontaneous miniature inhibitory postysynaptic currents (mIPSCs), the enhancement of the inhibitory charge attributable to the tonic conductance was several-fold greater than that mediated by mIPSCs. Finally, extrasynaptic $GABA_A$ receptors are expressed in brain regions that are involved in anesthetic-sensitive behaviors such as hippocampal-dependent memory and thalamic-dependent consciousness. Of particular interest are the actions of anesthetics on extrasynaptic $GABA_A$ receptors in two regions: the pyramidal neurons in the CA1 regions of the hippocampus and the thalamic VB neurons.

3. Memory impairment by anesthetics and tonic inhibition

The impairment of memory is one of the most potent effects of many general anesthetics (Campagna et al., 2003), and the dose of etomidate that impairs memory is considerably lower than the dose that causes immobility (Cheng et al., 2006). From a clinical perspective, the disruption of memory by anesthetics has gained substantial attention from the medical and lay community. Amnesia is one of the most important effects of anesthetics, yet some patients experience inadequate amnesia during anesthesia. This leads to the unexpected recall of unpleasant surgical events (Sebel et al., 2004). On the other end of the spectrum, some patients, particularly the elderly, experience persistent postoperative memory deficits (Hudetz et al., 2007; Moller et al., 1998). The mechanisms underlying unexplained intraoperative awareness and persistent postoperative memory impairment remain unknown.

Recently, attention has turned to $GABA_A$ receptors containing the α_5 subunit, as these receptors may contribute to the amnestic effects of anesthetics. In the hippocampal pyramidal neurons, tonic conductance is generated primarily by $GABA_A$ receptors that contain the α_5 subunit. Pharmacological studies suggest that this subunit combines with β_3 and γ subunits to form heteromeric complexes (Caraiscos et al., 2004a). The α_5 receptor subunit has a restricted pattern of distribution, being predominantly expressed in the hippocampal pyramidal neurons, where it is incorporated into approximately 20% of all $GABA_A$ receptors (Sur et al., 1999).

Several lines of evidence have implicated the α_5GABA_A receptor in learning and memory processes. Genetically modified mice lacking the α_5 subunit of the $GABA_A$ receptor because of chromosomal deletion ($\alpha5-/-$) exhibit better hippocampal-dependent learning than wild-type littermates (Collinson et al., 2002). In another knock-in mutant mouse model, expression of the $\alpha5$ subunit was unexpectedly reduced in mice expressing a point mutation of the α_5 subunit in which the histidine residue at position 105 was replaced with arginine (His105Arg; Crestani et al., 2002). These α_5His105Arg mice also had enhanced memory performance for trace fear conditioning. In addition, inverse agonists that selectively inhibit the activity of α_5GABA_A receptors improved memory performance in animal models (Chambers et al., 2003; Chambers et al., 2004) and in humans with ethanol-induced memory impairment (Nutt et al., 2007).

Studies using hippocampal slices and primary cultures of hippocampal neurons have shown that the tonic conductance generated by α_5GABA_A receptors in pyramidal neurons is enhanced by low (amnestic) concentrations of several classes of anesthetics, including propofol (Bieda and MacIver, 2004), isoflurane (Caraiscos et al., 2004b), and etomidate (Cheng et al., 2006). Furthermore, long-term plasticity of excitatory neurotransmission in hippocampal CA1 pyramidal neurons is widely considered to be a molecular substrate for memory. Long-term plasticity was reduced by etomidate in hippocampal slices from wild-type but not $\alpha5-/-$ mice (Cheng et al., 2006). Correlative behavioral studies showed that low, clinically-relevant doses of etomidate impaired the performance of wild-type but not α_5-subunit-deficient mice for hippocampal-dependent learning and memory tasks, including spatial navigation in the Morris water maze and memory for an adverse stimulus studied with Pavlovian contextual fear conditioning (Cheng et al., 2006; Fig. 2). Equally interesting is the similar impairment of motor coordination (measured using the rotarod), loss of righting reflex, and anxiolytic effects induced by etomidate in the two genotypes. Together, these results suggest that the α_5GABA_A receptor subunits mediate the memory-impairing effects of etomidate, but not the sedative or hypnotic effects of this drug. Most importantly, memory can be dissociated from other endpoints associated with the anesthetic state on the basis of genetic differences.

It is likely that the receptors contributing to the amnestic properties of etomidate contain β_2 and/or β_3 subunits, as etomidate is almost 10 times as efficacious at $GABA_A$ receptors containing these two types of subunits as it is at receptors containing the β_1 subunit (Hill-Venning et al., 1997). Replacing the asparagine residue at position 265 on either the β_2 or β_3 subunit with methionine greatly reduced the modulatory actions of etomidate in vitro (Siegwart et al., 2002). A recently developed radioactive, photosensitive etomidate derivate, [^{3}H] azietomidate, was used to directly label the residues surrounding the etomidate binding site (Li et al., 2006). The ability to directly label amino acids in the binding site is highly advantageous, since the identification a binding site using this method would be independent of allosteric effects of etomidate on channel gating. The methionine residue at position 286 on the β_3 subunit was photolabeled by [^{3}H]azietomidate, which confirmed in vitro data indicating the importance of the β

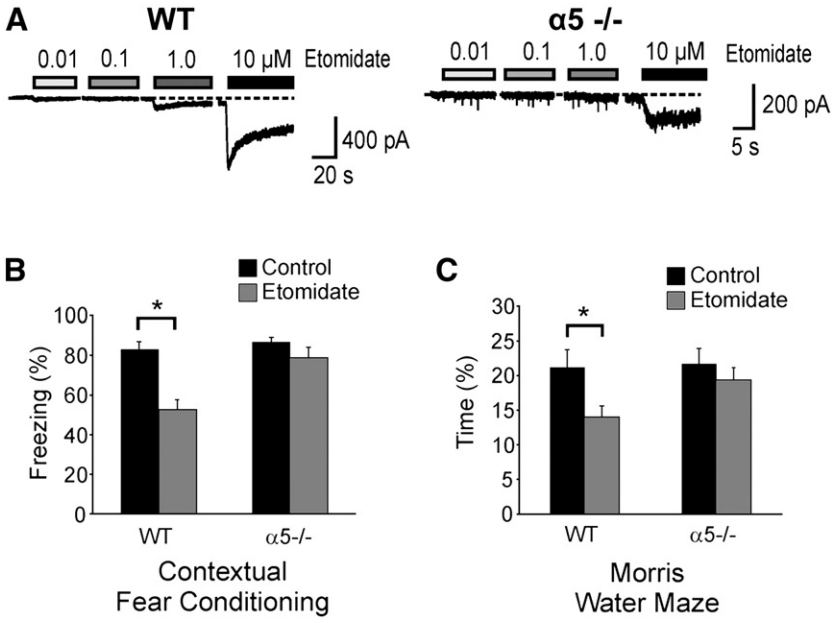

Fig. 2. Amnestic actions of etomidate, as mediated by α5 subunit-containing γ-aminobutyric acid subtype A receptors. Hippocampal CA1 pyramidal neurons exhibit a tonic current, which is largely mediated by α5-subunit-containing $GABA_A$ receptors. (A) At low concentrations, etomidate (<1 μmol/L) potentiates the tonic current in wild-type hippocampal neurons but not in neurons from mice lacking the α5 subunit (α5−/−). (B) Low doses of etomidate impair memory performance of wild-type but not α5−/− mice in hippocampal-dependent learning tasks. Reproduced with permission from Cheng et al., 2006.

subunit in etomidate binding. Surprisingly, a methionine residue on the α_1 subunit at position 236 was also labeled, which suggests that the α subunit contributes to the etomidate binding site. The photolabelling of these sites was predicted to also occur at homologous methionine residues on the β_1, β_2, α_2, α_3, α_5 subunits. These data suggest that etomidate occupies a water-filled pocket in the $GABA_A$ receptor near the GABA binding site.

4. Sedation and $GABA_A$ receptor isoforms

The terms sedation and hypnosis are sometimes used synonymously. However, here they are distinguished as recent studies suggest that different brain regions and receptor populations mediate sedation and hypnosis. Sedation in humans refers to a deceased level of arousal, as indicated by longer response times, decreased motor activity and slurred speech. In animal models, the surrogate measure for sedation is a reduction in motor activity and decreased arousal (Campagna et al., 2003). Hypnosis refers specifically to the loss of consciousness. In animal studies, the loss of the righting reflex is used as an indicator of the hypnotic state (Rudolph and Antkowiak, 2004). Ambiguity in the literature regarding the definition of sedation and hypnosis has lead to confusion. Consensus-based functional definitions for the various behavioral endpoints associated with general anesthesia need to be developed by the research community.

Although the amnestic properties of etomidate likely involve hippocampal α_5GABA_A receptors, the drug's sedative actions may depend on other $GABA_A$ receptor isoforms. Knock-in mutant mice have been used to differentiate the $GABA_A$ subunits responsible for the sedative and hypnotic effects of etomidate. Replacing the asparagine at position 265 in the β2 or β3 subunits with serine or methionine, respectively, renders $GABA_A$ receptors containing these subunits insensitive to etomidate in vitro (Jurd et al., 2003; Reynolds et al., 2003). In β_2(Asn265Ser) mice, low doses of etomidate failed to impair rotarod performance or reduce spontaneous locomotor activity, which implies that the sedative actions of etomidate depend on $GABA_A$ receptors containing the β_2 subunit (Reynolds et al., 2003).

Studies of benzodiazepines have implicated the α1 subunit in mediating the sedative property of diazepam. While pharmacologically distinct from general anesthetics, diazepam has pronounced sedative activity. $GABA_A$ receptors that contain a histidine to arginine mutation at position 101 of the α1 subunit were less sensitive to positive allosteric enhancement by diazepam in vitro (Rudolph et al., 1999). Behavioral studies showed that the sedative properties of diazepam were eliminated in knock-in mice that expressed the α1(His101Arg) mutation (Rudolph et al., 1999).

The α_1 and β_2 subunit-containing $GABA_A$ receptors in the neocortex are thought to contribute to the sedative actions of several inhaled anesthetics. These subunits are densely in the cortex, and with the γ_2 subunit comprise more than 40% of all $GABA_A$ receptors in the brain (McKernan and Whiting, 1996). $GABA_A$ receptors containing the $\alpha_1\beta_2\gamma_2$ subunits are enriched at synaptic sites throughout the brain (Farrant and Nusser, 2005). This suggests that the enhancement of synaptic activity within the neocortex could be responsible for anesthetic sedation. The contribution of the neocortex to the sedative properties of inhaled anesthetics was studied by Hentschke and colleagues (Hentschke et al., 2005). The depressive effects of isoflurane, enflurane and halothane on rat neocortical neuron

activity were studied using in vivo recordings of spontaneous action-potential firing and in vitro recordings from isolated cortical networks. Sedative concentrations of isoflurane, enflurane and halothane similarly reduced the firing of spontaneous action potentials in vivo and in vitro by approximately 50%. This reduction in neuronal firing strongly correlated with an increase in GABAergic synaptic inhibition. Anesthetics prolonged the time course of $GABA_A$ receptor-mediated spontaneous IPSCs from pyramidal neurons in organotypic cortical cultures with no effect on their frequency or peak amplitude. The authors conclude that $GABA_A$ receptors in the neocortex that generate synaptic inhibition are direct molecular targets for inhaled anesthetics and contribute to the sedative actions of isoflurane, enflurane and halothane.

The thalamus is a large mass of gray matter deeply situated in the forebrain that is critically involved in sleep and waking processes (Steriade, 2005). Synchronous electrical activity in the thalamaocortical neurons of the VB complex facilitates the transition from the awake and rapid eye movement sleep states to the "spindle mode" and "delta mode" that characterize light and deep sleep, respectively (Steriade, 2005). Tonic current in the thalamic VB neurons may contribute to the sedative action of THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol). Although THIP is not commonly used as an anesthetic, it promotes slow wave sleep and has analgesic, sedative, hypnotic, and ataxic properties (Krogsgaard-Larsen et al., 2004). $GABA_A$ receptors that contain the α4 and δ subunit appear to contribute to the sedative action of THIP. At low concentrations, THIP strongly potentiates the activity of $GABA_A$ receptors containing the δ subunit and enhances a tonic conductance generated by $\alpha_4\delta GABA_A$ receptors (Brown et al., 2002). Rotarod performance and spontaneous locomotor activity were unimpaired by THIP in α_4 subunit knockout mice (Chandra et al., 2006), which suggests that $\alpha_4\delta$ subunit-containing $GABA_A$ receptors are necessary for the sedative and ataxic effects of THIP.

The thalamic expression of β subunits is well differentiated, where the VB neurons express the β_2 subunit, and neurons in the nucleus reticularis (nRT) neurons predominantly express the β_3 subunit (Belelli et al., 2005). The VB but not the nRT neurons exhibit a large tonic conductance (Belelli et al., 2005). This inhibitory conductance has been shown to result from the presence of high-affinity $GABA_A$ receptors containing both α_4 and δ subunits in the VB neurons (Chandra et al., 2006). THIP enhanced the tonic but not the phasic current in VB neurons, and had no effect on nRT neurons (Reynolds et al., 2003). Since the sedative actions of THIP were absent in α_4 knockout mice, it is likely that the tonic current mediated by $\alpha_4\beta_2\delta$ $GABA_A$ receptors in VB neurons contributes to anesthetic sedation. This is in agreement with the finding that etomidate-mediated sedation also depends on $GABA_A$ receptors containing the β_2 subunit (Jurd et al., 2003; Reynolds et al., 2003), although the specific contribution of thalamic β_2 subunits to this effect is uncertain.

5. Hypnosis and $GABA_A$ receptor isoforms

Hypnosis typically requires higher concentrations of anesthetics than sedation (Rudolph and Antkowiak, 2004) and is often measured by the loss of the righting reflex in rodents. Anesthetic action on $GABA_A$ receptors is implicated in hypnosis. The duration of the loss of righting reflex induced by etomidate was reduced in β_2(Asn265Ser) mice (Reynolds et al., 2003; Fig. 3). Interestingly, the electroencephalographic

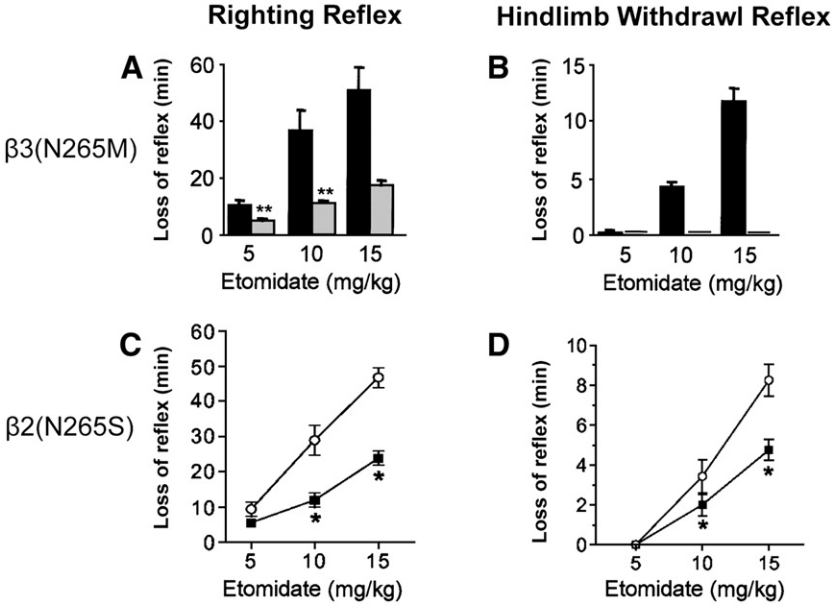

Fig. 3. Etomidate hypnosis and immobilization in β3(Asn265Met) and β2(Asn265Ser) mice. In β3(Asn265Met) mice, etomidate-induced hypnosis (loss of righting reflex) is greatly attenuated (A), and immobilization (loss of hind limb withdrawal reflex) is completely abolished (B). Reproduced with permission from Jurd et al., 2003. In β2(Asn265Ser) mice, etomidate-induced loss of righting reflex (C) and loss of hind limb withdrawal reflex (D) are also reduced, although the reduction in immobilization is less prominent than in β3(Asn265Met) mice. Reproduced with permission from Reynolds et al., 2003.

patterns during etomidate anesthesia were similar for β_2 (Asn265Ser) and wild-type mice, indicating that loss of consciousness is not mediated by the β_2 subunit (Reynolds et al., 2003). However, $GABA_A$ receptors containing the β_2 subunit are only partially responsible for the hypnotic effect of etomidate. Etomidate-induced hypnosis also depends on the β_3 subunit as the duration of loss of righting reflex after intravenous injections of etomidate was reduced in β_3 (Asn265Met) mice (Jurd et al., 2003; Fig. 3).

Anesthetic immobilization in rodents is often measured as the lack of a withdrawal reflex to noxious stimuli. Although this loss of a reflex is superficially similar to the hypnotic effects of anesthetics, the immobilizing actions of anesthetics are thought to arise from complex interactions of spinal and supraspinal inhibition (Rudolph and Antkowiak, 2004; Sonner et al., 2003). The immobilizing effect of etomidate, as measured by the hind limb withdrawal reflex, was similar in β_2(Asn265Ser) and wild-type mice (Reynolds et al., 2003) but were absent in β_3 (Asn265Met) mice (Jurd et al., 2003; Zeller et al., 2007). Together the results show that $GABA_A$ receptors containing the β_3 subunit are primarily responsible for immobilization by etomidate.

The specific brain regions targeted by anesthetics to produce hypnosis are not well defined. The effects of THIP on thalamic neuronal may contribute to the hypnotic properties of this drug (Belelli et al., 2005). The tubermomammillary nucleus (TMN) of the hypothalamus has also been implicated in anesthetic hypnosis. The duration of loss of righting reflex by propofol was substantially reduced by injecting the GABA antagonist gabazine directly into the TMN (Nelson et al., 2002).

6. Volatile anesthetics and $GABA_A$ receptor composition

Volatile anesthetics are low-potency compounds that influence a variety of receptors at clinically-relevant concentrations (Campagna et al., 2003). Thus, determining the specific sites of action that underlie the desirable therapeutic effects of volatile anesthetics is a challenge. In addition, behavioral testing with volatile anesthetics is difficult for practical reasons. Nevertheless, several carefully designed studies have suggested that several endpoints of isoflurane anesthesia are mediated by distinct subpopulations of $GABA_A$ receptors.

For many years, a binding site for volatile anesthetics at the $GABA_A$ receptor remained elusive. However, recent studies have identified a discrete anesthetic-binding cavity for volatile anesthetics that critically involves the α_1 subunit (Jenkins et al., 2001; Koltchine et al., 1999; Mihic et al., 1997). Using anesthetics of different molecular size (specifically, isoflurane, halothane, and chloroform) and complementary site-directed mutagenesis, a water-filled cavity that functions as a binding pocket for volatile anesthetics was proposed to exist on the $GABA_A$ receptor (Jenkins et al., 2001). Identification of this binding pocket overturned a long-held assumption that anesthetics acted through a nonspecific mechanism.

The identity of the amino acids involved in the activity of volatile anesthetics was probed with chimeric $GABA_A$ receptors constructed from native subunits and site-directed mutagenesis. Two amino acids in the α_1 subunit are critical for anesthetic action (Mihic et al., 1997): serine 270 in the transmembrane 2 region and alanine 291 near the extracellular regions of transmembrane 3. The substitution of larger amino acid residues at Ser270 in the α_1 subunit rendered the $GABA_A$ receptors less sensitive to volatile anesthetics (Koltchine et al., 1999; Nishikawa et al., 2002), whereas substituting smaller-volume residues had the opposite effect (Koltchine et al., 1999). Also, replacing the α_1 Ser270 residue with histidine yielded recombinant heteromeric $GABA_A$ receptors that were insensitive to isoflurane (Koltchine et al., 1999). However, the α_1 (Ser270His) mutation introduced an additional change to the $GABA_A$ receptors that complicated the interpretation of receptor pharmacology. In particular, the potency of GABA at the $GABA_A$ receptor was increased (Hall et al., 2004). This problem was addressed by introducing an additional mutation into the α_1 subunit, whereby the leucine residue at position 277 was replaced with alanine. This double knock-in mutation, α_1 (Ser270His,Leu277Ala), restored normal sensitivity to GABA (Borghese et al., 2006). These mutations laid the foundation for generating knock-in mice that were partially insensitive to isoflurane.

Mice that expressed a double knock-in mutation were generated to explore the contribution of $GABA_A$ receptors containing α_1 subunits to isoflurane anesthesia (Sonner et al., 2007). The righting reflex of double-mutant mice expressing the α_1(Ser270His,Leu277Ala) subunit was less impaired by isoflurane than was the case for wild-type controls, which suggested that the hypnotic actions of isoflurane are dependent on the α_1 subunit. However, the immobilizing effects of isoflurane as measured with the tail clamp withdrawal reflex were normal in the double knock-in mice. The amnestic action of isoflurane, measured with cued and contextual fear conditioning, was also similar in wild-type and α_1(Ser270His, Leu277Ala) mice, which suggested that this subunit is not important for isoflurane-induced amnesia. This last finding is in contrast to previous work using mouse mutants in which the α1 subunit was knocked out either globally or in the forebrain (Sonner et al., 2005). In these mice, the amnestic actions of isoflurane were reduced, indicating that the α1 subunit is involved in isoflurane amnesia.

The β subunit of $GABA_A$ receptors also contributes to the volatile anesthetic-binding site and the behavioral effects of volatile anesthetics (Mihic et al., 1997; Siegwart et al., 2002). Replacing methionine at position 286 with tryptophan in the β_1 subunit reduced the potentiating effects of isoflurane (Mihic et al., 1997). Additionally, enflurane potentiation of GABA current was reduced by substituting the asparagine residue at position 265 with methionine or the methionine at position 286 with tryptophan on the β_3 subunit (Siegwart et al., 2002). The behavioral effect of isoflurane interactions at the β_3 subunit were studied with β_3(Asn265Met) knock-in mice. Isoflurane is slightly less potent at inhibiting the righting reflex in β_3 (Asn265Met) mice (Lambert et al., 2005), which suggests that the β_3 subunit plays a small role in isoflurane hypnosis. However, the immobilizing effect of isoflurane, as measured by the hind limb (Lambert et al., 2005) or tail clamp withdrawal

reflex (Liao et al., 2005), is significantly reduced in these knock-in mice. In addition, isoflurane decreased heart rate and core temperature to a lesser degree in β_3(Asn265Met) mice, which indicates that the β_3 subunit has a role in these actions (Zeller et al., 2007). Thus, distinct $GABA_A$ receptor subunits contribute to the neurodepressive and cardiovascular effects of volatile anesthetics.

7. Conclusions

Our knowledge of how general anesthetics act on their targets to produce anesthesia has advanced considerably over the past decade. Genetic manipulations and subunit-selective drugs have been used to identify discrete $GABA_A$ receptor populations that are key mediators of anesthesia endpoints such as sedation, hypnosis, and amnesia. Additionally, the discovery of endpoints specific for certain $GABA_A$ receptors is furthering our understanding of how these subunits contribute to the biology of cognitive function. It is hoped that continuing progress in this direction will eventually yield a new generation of anesthetic drugs that can be used to selectively induce the desired anesthetic state without risk of broad neurodepression.

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